THE CHROMOSOMES OF *CHRYSANTHEMUM*, III: MEIOSIS IN *C. ATRATUM*

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I. INTRODUCTION

GERM CELLS with unreduced nuclei arise in plants and animals from irregularities in meiosis, owing to genotypic causes or to structural hybridity. Many types of upset in the meiotic cycle have been reported and we are now coming to know the kinds of errors by which these variations arise.

Genotypically controlled irregularities are often associated with reduced precocity of the early meiotic stages. A new type of irregularity, intermediate between normal meiotic and completely asynaptic behaviour, has now been found in *Chrysanthemum atratum*.

C. atratum exists in two forms, diploid $2n = 18$ and autotetraploid $2n = 36$. All nine chromosomes of the basic set have nearly median centromeres. Meiosis in the diploid form is normal. The meiotic abnormalities were found in four tetraploid plants, probably of one clone, obtained from the Royal Horticultural Society, Wisley. The similarity in behaviour of these plants suggests that they have arisen from a clone.*

2. MEIOSIS

Normal cells.—Pairing and chiasma formation in normal cells is like that in other autotetraploid species of *Chrysanthemum*. Associations of two, three and four chromosomes are observed with varying numbers of univalents; bivalents each had 1-3 chiasmata (fig. 1). Both first and second meiotic divisions follow the usual course, the univalents dividing at AI. There is the expected variation in chromosome number of the resultant pollen grains.

Precentric cells.—In 63 per cent. of pollen mother cells there were regularly 18 bivalents at MI. These were abnormal in that they showed a precocious splitting of their centromeres as well as a reduced chiasma frequency. In fact each had a single terminal chiasma. Cells behaving in this unusual way have been termed "precentric cells" and the chromosome associations they contain "precentric bivalents." Seven per cent. of cells were intermediate and had both

* A modified Feulgen technique was used for anther squashes with acetic alcohol (1:3) as fixative. A hydrolysis time of 4 mins. was sufficient as opposed to the 15 mins. for somatic root-tip squashes (Dowrick, 1952). Anthers were also fixed in a solution of absolute alcohol and saturated ferric acetate in glacial acetic acid (3:1) and stained in acetocarmine. Extra iron was unnecessary. Both methods gave satisfactory results.

normal and precentric chromosome associations in the same cell (table, fig. 3).

FIG. 1.—(a) First metaphase in a normal pollen mother cell of *C. atratum*. (b) First anaphase in a normal cell. $\times 1200$.

Abnormalities are confined to pollen mother cells: root-tip mitoses are normal. The abnormal divisions were present in similar proportions not only in all four plants, but also in all five anthers of

TABLE
Frequency of precentric pollen mother cells at MI

	Cells examined	Normal	Mixed	Precentric
Plant 1 . . .	91	26	3	62
Plant 2 . . .	79	23	7	49
Plant 3 . . .	52	18	4	30
Plant 4 . . .	107	31	10	66
Total . . .	329	98	24	207
Percentage . .	...	30	7	63

the individual florets (table). There is a random distribution of precentric cells. They are scattered throughout the anthers, both in groups and isolated.

The division of the centromeres does not occur until the bivalents are already on the metaphase plate. The arrangement of the bivalents is then normal. Their appearance, with divided centromeres and completely terminalised chiasmata, is quite characteristic (fig. 2 and Plate).

FIG. 2.—(a) and (b) first metaphase in *precentric* cells. (c) First anaphase with precociously split chromosomes resulting from such metaphases. $\times 1200$.

The half-bivalents separate from each other normally, each passing to its respective pole without active repulsion between the divided centromeres of each chromosome. However, having already divided precociously, the centromeres of the chromatids tend, during anaphase movement, to fall apart with the result that at first telophase each nucleus contains 36 chromosomes. The first meiotic division of the abnormal cells does not, therefore, reduce the chromosome number. Nor, since crossing over is near the ends, can it greatly change the genetic constitution of the cell.

In normal cells at the conclusion of first division the chromosomes are, with the exception of the centromere, structurally double. But, as they have single centromeres, they are mechanically single. Consequently, at MII, when the centromeres divide and the two halves of each chromosome separate, the number of centromeres in each daughter cell is half that in the mother cell.

In precentric pollen mother cells, however, where the chromosomes have divided at MI and the half chromosomes have separated at AI,

the interphase chromosome threads are single throughout. They are similar to daughter univalents and, like them, do not divide again during a second meiotic division (Darlington, 1939). Where this applies to all the chromosomes it appears that the second division of the nucleus itself is precluded. This observation lends support to the view that the occurrence of a second meiotic division is determined, or precipitated, by the undivided nature of the centromere at the end of first division. Otherwise it might be expected that a second division would be found in the present case. In fact meiosis ends with the formation of dyads, the cells of which retain the somatic chromosome number. Both diploid and tetraploid pollen grains are therefore found in the same anther.

FIG. 3.—First metaphase in cell with both normal and precentric chromosome associations.
× 1600.

In the 7 per cent. of cells with mixed chromosome associations their proportions vary (fig. 3). I have been unable to trace their later history but the interphase nuclei arising from them will contain both single and double chromosome threads and, therefore, no doubt, be capable of undergoing a second division. The daughter chromosomes from the precentric bivalents will, presumably, be scattered through the four resultant nuclei.

In 3 of the 207 precentric cells (table), there were bivalents with chiasmata at both ends. Their behaviour is similar to those with a single chiasma.

In normal cells, where pairing and coiling are complete, the chiasmata are situated near the chromosome ends. Pairing must, therefore, start at the ends if, as has been suggested previously by Darlington (1935), the degree and distribution of pachytene pairing and coiling determine the number and position of chiasmata. It must then be assumed that, in the precentric cells of *C. atratum*, the paired chromosomes usually pair and coil at only one end and consequently only a single terminal chiasma is formed.

3. THE ORIGIN OF THE ABNORMALITY

These peculiarities of meiosis arise, as we should expect, before first metaphase. The precentric cells come into prophase later, for while normal cells are at diplotene the abnormal cells are still in a pre-pachytene condition. Later they catch up and both types of

FIG. 4.—The two types of meiosis in pollen mother cells of *C. atratum*. The reduced pairing and precociously split centromeres in precentric cells results in the formation of dyads.

cell enter first metaphase together (fig. 4). Pachytene and later prophase stages must, therefore, progress more rapidly than in normal cells. Chromosome pairing and relational coiling in the precentric cells is, it is assumed, less complete than in the normal cells. This will result in a reduced chiasma frequency and, if coiling starts at the chromosome ends, more complete terminalisation. It will not, however, explain the precocious division of the centromeres.

FIG. 5.—The relative sizes of normal and precentric cells and nuclei.

Correlated with these timing abnormalities are significant size differences between the different cell types. At early prophase, pollen mother cells which will show an irregular meiosis are more than twice as large as normal cells while their nuclei are approximately four times as large (fig. 5). This size advantage of the precentric cells is retained throughout the first division. Hence mature pollen grains arising from dyads have a volume, not twice, but more than three times as great as normal grains.

4. DISCUSSION

The abnormalities in *C. atratum* are, presumably, brought on by the genetic control of physiological differences in the cells at the onset of meiosis.

C. atratum and the parthenogenetic earthworms described by Muldal (1952), form a special group, in regard to abnormal meiosis, where there is precocious splitting of the centromeres and the production of unreduced gametes. This precentric behaviour is intermediate between normal meiosis and "asynapsis" where chiasmata are absent and only univalents are found. In all other respects the abnormality in *C. atratum* is analagous to that found in

Antennaria (Stebbins, 1932), *Hieracium* (Gustafsson, 1942), *Calamagrostis* (Nygren, 1946) and *Scilla* (Rees, 1952).

The similarities are :—

- (i) Only some germ cells, not all, in each anther are affected.
The proportion of abnormal cells varies from as little as 1 per cent. in some samples of *Scilla* to 63 per cent. in *C. atratum* and 99 per cent. in *Datura*.
- (ii) The distribution of abnormal cells is always at random.
- (iii) Prophase begins later in abnormal cells, metaphase sometimes, but not always, catching up.
- (iv) There is increase in the size of the retarded cells in relation to the normal.

Male sterile *Lathyrus odoratus* (Upcott, 1937) is, like *C. atratum*, also intermediate between asynapsis and normal meiosis. It is also similar in that prophase in abnormal cells begins later. It differs in being a total defect of all cells and in not being precentric. The delay in early meiosis brings about, in this case, a greater terminal localisation of the chiasmata at first metaphase. Carried to its extreme this increased localisation would result, as in *Scilla*, in asynapsis.

The behaviour of E.M.C's. in these various plants, may be similar to, or different from, the pollen mother cells. The cells arising from the abnormal divisions may either give rise to fertile gametes or, in other cases, degenerate at varying stages before reaching maturity. In *Antennaria*, *Hieracium* and *Calamagrostis*, meiosis may follow yet a third course : it may be replaced by mitosis in the abnormal pollen mother cells and embryo sacs.

Many other examples of asynapsis are known at meiosis in plants where the properties that are discussed here are not described, as in *Datura* (Satina and Blakeslee, 1934) and *Pyrus* (Thomas, 1942).

5. SUMMARY

1. An autotetraploid clone of *C. atratum* has unreduced pollen as a result of abnormal meiosis in 68 per cent. of pollen mother cells.
2. The abnormal cells lag at prophase, have a prolonged growth phase, and are less precocious in chromosome development than normal cells. They are consequently larger in both cell and nuclear size throughout all stages of division.
3. In these cells no quadrivalents are formed. The bivalents show a reduced chiasma frequency and precocious splitting of the centromeres at MI. The chromatids fall apart during anaphase.
4. The first division gives rise to cells with 36 chromosomes. A second division is omitted. Thus, it appears, it is the capacity of the centromeres for division which determines or precipitates the occurrence of the second meiotic division.

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Plate

1. Normal first metaphase with four quadrivalents and ten bivalents. $\times 2600$.
2. First metaphase in precentric cell with eighteen bivalents and precociously divided centromeres. $\times 2600$.
3. Two normal first anaphases with eighteen daughter bivalents in each. $\times 1000$.
4. First anaphase in a precentric cell ; 36 chromosomes passing to each pole. $\times 1000$.

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